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## Dietary manipulation reveals an unexpected inverse relationship between fat mass and adipose 11 $\beta$ -hydroxysteroid dehydrogenase type 1

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### Abstract

Increased dietary fat intake is associated with obesity, insulin resistance, and metabolic disease. In transgenic mice, adipose tissue-specific overexpression of the glucocorticoid-amplifying enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) exacerbates high-fat (HF) diet-induced visceral obesity and diabetes, whereas 11 $\beta$ -HSD1 gene knockout ameliorates this, favoring accumulation of fat in nonvisceral depots. Paradoxically, in normal mice HF diet-induced obesity (DIO) is associated with marked downregulation of adipose tissue 11 $\beta$ -HSD1 levels. To identify the specific dietary fats that regulate adipose 11 $\beta$ -HSD1 and thereby impact upon metabolic disease, we either fed mice diets enriched (45% calories as fat) in saturated (stearate), monounsaturated (oleate), or polyunsaturated (safflower oil) fats ad libitum or we pair fed them a low-fat (11%) control diet for 4 wk. Adipose and liver mass and glucocorticoid receptor and 11 $\beta$ -HSD1 mRNA and activity levels were determined. Stearate caused weight loss and hypoinsulinemia, partly due to malabsorption, and this markedly increased plasma corticosterone levels and adipose 11 $\beta$ -HSD1 activity. Oleate induced pronounced weight gain and hyperinsulinemia in association with markedly low plasma corticosterone and adipose 11 $\beta$ -HSD1 activity. Weight gain and hyperinsulinemia was less pronounced with safflower compared with oleate despite comparable suppression of plasma corticosterone and adipose 11 $\beta$ -HSD1. However, with pair feeding, safflower caused a selective reduction in visceral fat mass and relative insulin sensitization without affecting plasma corticosterone or adipose 11 $\beta$ -HSD1. The dynamic depot-selective relationship between adipose 11 $\beta$ -HSD1 and fat mass strongly implicates a dominant physiological role for local tissue glucocorticoid reactivation in fat mobilization.

### Keywords

glucocorticoid; dietary fats

### Introduction

Specific dietary fats affect insulin sensitivity and consequently have a major impact upon metabolic disease over and above their contribution to total calorie content (39, 49). Stearate, a long-chain saturated dietary fat (18:0), directly inhibits insulin signaling in

### Disclosures

The authors declare that they have no competing interests.

muscle cells, whereas long-chain unsaturated fats (oleate 18:1) and shorter-chain saturated fats do not (8). Oleate enrichment can improve insulin sensitivity if overall fat content is low (49), whereas polyunsaturated fats improve insulin sensitivity (20).

High glucocorticoid levels (e.g., Cushing's syndrome) cause insulin resistance and promote visceral fat accumulation, whereas adrenalectomy ameliorates obesity in rodents (18, 22). Substantial evidence links the disturbances of fatty acid and glucocorticoid metabolism in obesity. Fatty acids and high-fat diets can cause adrenal and pituitary activation in rodents (15, 42, 45, 53), suggesting that in the obese/diabetic state, a feed-forward effect of high-plasma free fatty acids and increased adrenal glucocorticoid production may exacerbate insulin resistance. Moreover, saturated fats and glucocorticoids may induce insulin resistance in tissues through a common ceramide-mediated mechanism (14).

Human idiopathic obesity is associated with high intra-adipose glucocorticoid levels generated by the intracellular enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) rather than high circulating glucocorticoids (31). Transgenic overexpression of 11 $\beta$ -HSD1 in mouse adipose tissue causes insulin-resistant diabetes, dyslipidemia, and hypertension (23, 24). 11 $\beta$ -HSD1-deficient mice resist high-fat diet-induced obesity (DIO) due to beneficial changes in fat distribution and insulin sensitivity of adipose tissue and liver (28, 29). Similar protective effects are found in mice overexpressing the glucocorticoid-inactivating enzyme 11 $\beta$ -HSD2 specifically in fat (16).

Since obesity in humans and monogenic rodent models is associated with high adipose 11 $\beta$ -HSD1 levels (31), it was unexpected that DIO caused a pronounced adipose-specific downregulation of 11 $\beta$ -HSD1 in rodents (11, 30). Furthermore, DIO-resistant mouse strains shut down adipose 11 $\beta$ -HSD1 expression to a greater extent than DIO-prone strains (27, 30), suggesting that this response might be an intrinsic mechanism invoked to counter insulin resistance.

Despite these findings, little is known about the impact of individual fatty acids upon tissue level glucocorticoid action and the cross-talk between these two key contributors to fat storage and insulin resistance. To address this, we selectively enriched dietary long-chain saturated, monounsaturated, and polyunsaturated fatty acids and investigated their effects on fat mass, adipose, and liver tissue 11 $\beta$ -HSD1 and glucocorticoid receptor (GR) levels in mice.

## Methods

### Animals and diets

All experiments were approved by The University of Edinburgh ethics committee and were carried out according to the UK Animals (Scientific Procedures) Act of 1986. Age- and weight-matched C57BL/6J mice were housed in standard conditions on a 12:12-h light-dark cycle, lights on at 7 AM, and were randomly assigned to the diets. The experiment used singly housed adult age-matched (8 wk) male mice (n = 6) given one of the following diets (Research Diets, New Brunswick, NJ): control diet (11% calories as fat with cornstarch; D12329), a stearate-enriched diet (stearate; 45% calories from ethyl stearate, 100%; D04070901), an oleate-enriched diet (oleate; 45% calories from high oleic acid, ~80% C18:1; D04070902), and a safflower oil-enriched diet (safflower; 45% calories as safflower oil, ~80% C18:2; D04070903). A positive control for the previously observed adipose 11 $\beta$ -HSD1 downregulation was used (58HF; 58% calories as fat, mainly hydrogenated coconut oil: 99.1% saturated with 7.7% C8, 5.9% C10, 47.6% C12, 18% C14, 8.7% C16, 10.6% C18, and traces of unsaturated fats at ~0.8% C18:1 and 0.03% C18:2; D12331). A further positive control that was matched to the singly enriched diets was used in that 45% of the

calories consisted of fat (45HF: 45% calories with hydrogenated coconut oil, with the same composition as the 58HF; D04070907). All diets contained the same amount of soyabean oil (4% calories as fat; 14.2% saturated with 10.4% C16 and 3.8% C18, 24.3% monounsaturated, C18:1 and 61.3% polyunsaturated with 53.5% 18:2, and 7.8% C18:3). For the pair-feeding experiment, the three singly enriched diets (stearate, oleate, and safflower) and a low-fat control diet that was enriched with sucrose (D12328) were used. Ad libitum consumption was monitored for 1 wk, and the low-fat control was found to be the limiting diet for calorie intake in this run-in period. Therefore, all other diets were provided as calorie-matched amounts with respect to measured food intake daily before lights out (7 PM) and the amount adjusted to the continuously monitored average daily intake for mice on the control diet throughout (starting intake:  $0.23 \pm 0.008$  kcal·g body wt<sup>-1</sup>·day<sup>-1</sup>; final intake:  $0.39 \pm 0.08$  kcal·g body wt<sup>-1</sup>·day<sup>-1</sup>). Note that calorie-matching diets by measured food intake of the limiting diet, although it is standard practice, cannot control for loss of calories through malabsorption. Results pertaining to this unexpected effect of the stearate diet are described in context in the results and discussion.

### Tissue and plasma measurements

At the end of the experiment, mice were euthanized within 1 min of disturbing the home cage by cervical dislocation to avoid stress-induced changes in corticosterone. Blood was collected in EDTA-coated tubes (Sarstedt, Numbrecht, Germany). Plasma corticosterone was measured using an in-house RIA as described (28), insulin and leptin by ELISA (Crystal Chem, Downers Grove, IL), and glucose colorimetrically (Infinity reagent; Sigma, Dorset, UK). Liver, kidney, and epididymal, subcutaneous (sc), and mesenteric adipose tissues were collected, weighed, and stored at  $-80^{\circ}\text{C}$ .

### Gene expression

RNA was extracted using 800  $\mu\text{l}$  of TRIzol reagent (Invitrogen, Paisley, UK) per 50 mg of tissue. Briefly, 200  $\mu\text{l}$  of chloroform was added to the homogenate and then centrifuged at 12,000 rpm for 1 min to remove cell debris. The supernatant was then vortexed for 15 s and centrifuged at 12,000 rpm for 15 min at  $4^{\circ}\text{C}$ . The upper (aqueous) layer was removed, mixed with 30  $\mu\text{l}$  of RNAid+ matrix (Anachem, Luton, UK), and agitated for 5 min before centrifugation at 12,000 rpm for 1 min. The supernatant was removed and washed three times with 500  $\mu\text{l}$  of RNA wash (Anachem), resuspended in 20  $\mu\text{l}$  of diethyl pyrocarbonate-treated water with 10 mM DTT and 1 U/ml RNasin, and eluted by incubation at  $55^{\circ}\text{C}$  for 10 min. Concentration and purity of RNA were assessed using a GeneQuant RNA/DNA calculator before Northern blot or real-time PCR analysis. For Northern blotting, 5  $\mu\text{g}$  of RNA was denatured at  $65^{\circ}\text{C}$  for 15 min in a mixture of MOPS, deionized formamide, and formaldehyde as described (28) and run on denaturing MOPS-formaldehyde 0.8% agarose gels. Briefly, RNA was transferred using capillary action on a nylon membrane for hybridization, using  $20\times$  saline-sodium citrate (SSC) as the transfer buffer. RNA was cross-linked by UV exposure (power at  $1,200 \times 100 \mu\text{W}/\text{cm}^2$ ; Spectronics). Membranes were prehybridized at  $65^{\circ}\text{C}$  for 3 h with 18 ml of phosphate buffer and 9 ml of 20% SDS in a hybridization bottle. One milliliter of salmon testes DNA (10 mg/ml) was denatured at  $100^{\circ}\text{C}$  for 10 min and added to the prehybridization mix. Radiolabeled cDNA probes were made for genes of interest using a Rediprime 2 random prime labeling kit (Amersham). Twenty-five nanograms of DNA template (PCR fragment) was diluted to 45  $\mu\text{l}$  with Tris-EDTA buffer. The probe was then denatured at  $100^{\circ}\text{C}$  for 15 min and immediately cooled on ice for an additional 10 min. Denatured DNA was then added to the reaction tube together with 5  $\mu\text{l}$  of [ $^{32}\text{P}$ ]dCTP. The reaction was incubated at room temperature for 2 h, and then labeled cDNA was purified through a Nick column (GE Healthcare, Buckinghamshire, UK). The hybridization mixture was then incubated overnight at  $55^{\circ}\text{C}$ . Three 15-min, 50-ml washes were performed; an initial wash at room temperature with  $2\times$

SSC and 0.1% SDS was followed by two washes at 65°C with 1× SSC and 0.1% SDS and then 0.5× SSC and 0.1% SDS. The washed membrane was wrapped in Saran wrap and exposed to a phosphorimager screen (Fuji imaging plate) for 10 min and scanned using a Fuji BAS phosphorimager. Transcript levels were quantified using Aida software (Advance Image Data Analyzer, version 3.44.035).

### Real-time PCR

cDNA was synthesized from 2 µg of RNA using the Reverse Transcription system (Promega, Southampton, UK) with oligo(dT) primer according to the manufacturer's instructions. Liver GR mRNA levels were measured using the LightCycler 480 Real-Time PCR system (Roche Diagnostics, West Sussex, UK) with light cycler 480 probe master (Roche Diagnostics) and TaqMan Gene expression Assays (Applied Biosystems, Cheshire, UK). Samples were analyzed in triplicate with each PCR reaction containing 4.5 µl of cDNA, 5 µl of master mix, and 0.5 µl of primer/probe. Results are expressed as the ratio of liver GR to actin and/or TATA box-binding protein mRNA levels, which act as internal controls. TaqMan Gene expression Assays were nuclear receptor subfamily 3, group C, member 1 (mouse GR) Mm00433832\_m1, actin,  $\beta$ -cytoplasmic (actin) Mm00607939\_s1, and TATA box-binding protein, mouse 11 $\beta$ -HSD1 Mm00476182.

### 11 $\beta$ -HSD enzyme activity assays

11 $\beta$ -HSD1 is bidirectional in homogenates in vitro, with oxidative (dehydrogenase) more stable than dehydrogenase activity (19). Hence, dehydrogenase activity was measured routinely, which in the linear range of product formation gives an accurate reflection of total 11 $\beta$ -HSD1 protein levels. Tissues were homogenized in 10% glycerol, 300 mM sodium chloride, 1 mM EDTA, and 50 mM Tris, pH 7.7, and activity was measured as described (15). The protein concentration of each homogenate was determined colorimetrically by the Bradford method, using the Bio-Rad assay kit with bovine serum albumin protein standards (Bio-Rad, Hemel Hempstead, UK). Briefly, reactions (160 µl) containing 0.2 mg/ml protein, 10 nM [3H]corticosterone, and an excess (400 µM) of nicotinamide adenine dinucleotide phosphate with homogenization buffer were incubated in a shaking water bath at 37°C. After 1 h (adipose) or 10 min (liver) of incubation, steroids were extracted with ethyl acetate, separated by thin-layer chromatography, identified by comparison with the migration of unlabeled corticosterone and 11-dehydrocorticosterone standards, and quantified with a phosphorimager tritium screen (Fujifilm, Tokyo, Japan). Under these conditions, product formation was linear with respect to protein concentration and time (data not shown).

### Fecal fat extraction

Mice (n = 6) were housed individually in metabolic cages, and fecal matter was collected over a 1-wk period. Feces were dried at 40°C for 72 h in a preweighed 35-ml glass tube. Feces were sonicated in 1 ml water/0.1 g feces and rehydrated for 24 h. Chloroform-methanol (2:1) was added to the mixture (1:1), the homogenate was vortexed and left for 48 h, and layers were separated by centrifugation for 10 min at 2,000 g. Duplicate 2.5-ml aliquots of the organic layer were removed into preweighed fresh glass 12-mm tubes and dried under nitrogen. Fecal fat was assessed gravimetrically and presented as a percentage of the dry weight of the feces.

### Statistical analysis

Data were analyzed by one-way ANOVA using the SigmaStat program (Systat software) with post hoc Holm-Sidak tests to determine significance for multiple comparisons at a

threshold of  $P < 0.05$ . Linear regression and multiple linear regression were used to determine correlations.

## Results

### Effects of dietary fats on weight gain, food intake, and fat mass

Ad libitum feeding with stearate for 4 wk led to pronounced weight loss (starting weight:  $25.9 \pm 0.4$  g; final weight:  $22.9 \pm 0.7$  g), although caloric intake was higher than for all other diets (Fig. 1A). Monounsaturated oleate (starting weight:  $25.4 \pm 0.6$  g; final weight:  $31.5 \pm 0.9$  g) and, to a lesser extent, polyunsaturated safflower oil (starting weight:  $25.6 \pm 0.8$  g; final weight:  $29.0 \pm 1.0$  g) led to weight gain, although there was no difference in caloric intake. The most pronounced weight gain was with the 58HF (starting weight:  $24.3 \pm 1.0$  g; final weight:  $31.6 \pm 0.7$  g), which was included as a positive control for DIO-mediated 11 $\beta$ -HSD1 downregulation (30). Mice fed 45HF gained less weight (starting weight:  $24.8 \pm 0.6$  g; final weight:  $27.6 \pm 0.8$  g) but consumed a similar number of calories compared with those fed the 58HF diet. The 45HF diet had the same mixture of fats as the 58HF diet but had a lower overall percent fat content that matched the other diets. Adipose tissue weights correlated with body weight (Fig. 1B) and with plasma leptin levels ( $P < 0.001$ ,  $r > 0.71$ ). Oleate, 45HF, and 58HF caused small but significant increases in liver weight (Fig. 1B).

An additional pair-feeding experiment was carried out to determine whether altered food intake and, therefore, total calorie intake could account for the dietary fat-dependent differences in adiposity. All diets were calorie matched to the low-fat control (see METHODS). With this paradigm (Fig. 1, D–F), stearate caused a pronounced and significant body weight (starting weight:  $23.1 \pm 0.4$  g; final weight:  $19.66 \pm 0.7$  g) and fat mass loss (ANOVA,  $P < 0.01$  all fat depots). Mice fed the safflower diet did not gain weight (starting weight:  $23.6 \pm 0.4$  g; final weight:  $25.4 \pm 0.4$  g) and showed a reduction in mesenteric fat pad weight (Fig. 1D). Oleate increased weight gain (starting weight:  $21.9 \pm 0.3$  g; final weight:  $24.3 \pm 0.6$  g) but not fat pad weight (Fig. 1D). Fasting insulin correlated positively with fat mass ( $P < 0.001$ ) as expected (Fig. 1C).

Given the unexpected weight loss with stearate despite the highest caloric intake we tested for malabsorption. Fecal fat was higher in the stearate fed group ( $30.4 \pm 2.8\%$  of fecal dry weight,  $P < 0.001$  vs. 45HF and control) compared with control diet ( $0.8 \pm 0.02\%$  of fecal dry weight) and the comparator 45HF diet ( $7.8 \pm 0.7\%$  of fecal dry weight), consistent with stearate malabsorption.

### Effects of dietary fats on glucose homeostasis

To assess the effects of the diets on glucose homeostasis, we measured fasting glucose and insulin levels in the ad libitum-fed groups (Fig. 1D). Note that, although insulin sensitivity was not formally measured in our studies, the glucose/insulin ratio was entirely consistent with the expected insulinemic state found with the respective dietary effects on insulin sensitivity and the altered fat mass (8, 20, 39, 49).

Stearate caused relative hypoglycemia and hypoinsulinemia, whereas safflower oil and 45HF caused moderate fasting hyperglycemia. Oleate and 58HF caused marked fasting hyperglycemia and hyperinsulinemia, consistent with impaired glucose homeostasis and insulin resistance (Fig. 1D). Body weight was strongly correlated with fasting plasma insulin levels ( $P < 0.001$ ,  $r = 0.78$ ).

With pair-feeding, all mice ate their calorie-matched food parcels within a few hours of lights out. Therefore, to avoid a potentially confounding prolonged fast we measured morning glucose/insulin without a further defined fasting period. Glucose levels were



unchanged between the groups (Fig. 1G). Plasma insulin was still markedly reduced in the stearate group, whereas the hyperinsulinemia caused by ad libitum oleate feeding was corrected (Fig. 1G). Safflower oil caused a significant reduction in insulin levels (Fig. 1G) rather than the moderate increase seen with ad libitum feeding (Fig. 1D).

### Effects of dietary fats on plasma corticosterone level

Ad libitum feeding of oleate, safflower, 45HF, and 58HF diets for 4 wk suppressed nadir (morning) plasma corticosterone levels compared with control diet (Fig. 2A). In contrast, stearate significantly increased (2-fold) plasma corticosterone levels (Fig. 2A). There was a weak negative correlation of body weight with morning plasma corticosterone ( $P = 0.044$ ,  $r = -0.31$ ).

Pair-feeding accentuated the effect of stearate, causing a pronounced (~7-fold) increase in corticosterone (Fig. 2B). Oleate maintained significant suppression of corticosterone levels, but safflower had no effect (Fig. 2B).

### Effects of dietary fats on adipose 11 $\beta$ -HSD1 levels

With ad libitum feeding, stearate increased adipose 11 $\beta$ -HSD1 mRNA and activity levels in subcutaneous adipose tissue (Fig. 3, A and B). In contrast, all other (45% calories as fat) high-fat diets caused a marked and comparable reduction in adipose 11 $\beta$ -HSD1 mRNA levels in sc adipose tissue (Fig. 3A). Notably, 58HF caused a greater suppression of subcutaneous 11 $\beta$ -HSD1 activity (Fig. 3B) than the other high-fat diets.

Effects on mesenteric adipose were less pronounced; only 45HF and 58HF diets caused significant and similar reductions in adipose 11 $\beta$ -HSD1 mRNA levels (Fig. 3C). 58HF caused a pronounced downregulation of mesenteric adipose 11 $\beta$ -HSD1 activity (Fig. 3D), again disproportionately greater than its effect on 11 $\beta$ -HSD1 mRNA levels (Fig. 3C). Mesenteric adipose 11 $\beta$ -HSD1 activity (Fig. 3D) was threefold higher with stearate despite no significant effect on 11 $\beta$ -HSD1 mRNA (Fig. 3C).

Across all diets, body weight was strongly and negatively correlated with 11 $\beta$ -HSD1 activity in both subcutaneous ( $P < 0.001$ ,  $r = -0.67$ ) and mesenteric adipose tissue ( $P < 0.001$ ,  $r = 0.57$ ). Consistent with this being due to altered fat mass (Fig. 1B), leptin levels (in ng/ml: control  $4.7 \pm 0.7$ , stearate  $1.4 \pm 0.3$ , oleate  $14.2 \pm 1.9$ , safflower  $10.1 \pm 2$ , 45HF  $6.4 \pm 1.3$ , 58HF  $19.7 \pm 2$ ) strongly and negatively correlated with subcutaneous ( $P < 0.001$ ,  $r = 0.65$ ) and mesenteric 11 $\beta$ -HSD1 activity ( $P < 0.001$ ,  $F = 15.512$ ). Adipose mass showed a negative correlation with 11 $\beta$ -HSD1 activity in sc ( $P < 0.001$ ,  $r = -0.57$ ) but not mesenteric fat ( $P = 0.097$ ,  $r = -0.26$ ).

Notably, in the ad libitum feeding experiment, multiple linear regression showed that fasting insulin was strongly negatively correlated with sc ( $P < 0.001$ ,  $r = -0.54$ ) and mesenteric 11 $\beta$ -HSD1 activity ( $P = 0.012$ ,  $r = -0.44$ ).

With pair-feeding, sc adipose 11 $\beta$ -HSD1 mRNA levels (Fig. 4A) and activity (Fig. 4B) were increased with stearate and suppressed with oleate (Fig. 4, A and B), whereas safflower had no effect. As with ad libitum feeding, increased mesenteric adipose 11 $\beta$ -HSD1 activity (Fig. 4D) but not mRNA levels were observed with stearate, highlighting the discordance seen with ad libitum feeding in this depot (Fig. 3). Oleate and safflower had no effect on mesenteric adipose 11 $\beta$ -HSD1 (Fig. 4, C and D).

Linear regression showed corticosterone, but not insulin, correlated with sc ( $P = 0.013$ ,  $r = 0.81$ ) and mesenteric 11 $\beta$ -HSD1 activity ( $P = 0.007$ ,  $r = 0.76$ ) with pair-feeding.

To test whether changes in adipose 11 $\beta$ -HSD1 might precede and drive weight change, we performed a 1-wk ad libitum feeding experiment. Body weight did not change significantly (stearate before:  $23.1 \pm 0.8$  g; stearate after:  $20.1 \pm 1.2$  g; 45HF before:  $20.6 \pm 0.7$  g; 45HF after:  $20.9 \pm 0.6$  g) over 1 wk, as in our original study. Stearate caused a small but significant increase in subcutaneous but not mesenteric adipose 11 $\beta$ -HSD1 activity (Fig. 5). Similarly, 45HF caused a small but significant reduction in sc but not mesenteric adipose tissue (Fig. 5).

### Effects of dietary fats on adipose GR mRNA levels

A major determinant of glucocorticoid action is GR density. There was no significant effect of any diet on sc or mesenteric adipose GR mRNA with ad libitum or pair-feeding (data not shown).

### Effects of dietary fats on hepatic 11 $\beta$ -HSD1 and GR levels

Altered hepatic 11 $\beta$ -HSD1 (28, 36) and GR (34) affect glucose homeostasis. Therefore, we determined the effects of dietary fats on indices of glucocorticoid action in liver. Ad libitum feeding of 45HF and 58HF diets increased hepatic 11 $\beta$ -HSD1 mRNA levels (Fig. 6A). However, there was no effect of any diet on either hepatic 11 $\beta$ -HSD1 activity (Fig. 6B) or GR mRNA levels (data not shown).

## Discussion

Our data show that the changes in fat mass caused by distinct dietary fat enrichment associate specifically with changes in adipose tissue but not liver 11 $\beta$ -HSD1 activity. Although causation cannot be implied from associations, we show that changes in subcutaneous fat 11 $\beta$ -HSD1 precede weight change with short-term diet exposure. Moreover, the strong inverse correlations between fat mass and adipose 11 $\beta$ -HSD1 activity, rather than corticosterone, suggest that this local tissue mechanism of glucocorticoid regeneration is dominant over the HPA axis in fat mass regulation in response to dietary manipulation (15, 42, 45, 53). As discussed below, this is based on the known effects of glucocorticoids to drive lipolysis or fat accretion in the appropriate nutritional context. Moreover, the strong negative correlation of insulin with adipose 11 $\beta$ -HSD1, particularly in subcutaneous fat, vs. the strong positive correlation between corticosterone with adipose 11 $\beta$ -HSD1, particularly in visceral (mesenteric) fat, indicates a potential mechanistic basis for the depot-specific physiological regulation of fat mass through local glucocorticoid regeneration.

Elevated adipose 11 $\beta$ -HSD1 activity drives insulin resistance and metabolic disease (31). Therefore, we hypothesized that DIO-mediated downregulation of adipose 11 $\beta$ -HSD1 was a beneficial response counteracting insulin resistance in adipose tissue (11, 27, 30). However, the current findings challenge this view. First, of the two unsaturated fats, oleate, was the most potent in increasing fat mass and insulin resistance but also in suppressing corticosterone and adipose 11 $\beta$ -HSD1. Second, pair-feeding of the safflower diet accentuated its relatively (39) protective metabolic effects by reducing mesenteric fat mass and preserving apparent insulin sensitivity while failing to suppress corticosterone and adipose 11 $\beta$ -HSD1.

The most pronounced effect on weight gain and indices of glucocorticoid action was seen with the 58HF diet, which was used as a positive control to recapitulate our original observations (30). 58HF and the lower 45% HF diets contain almost exclusively saturated fats (99%), but these are of mixed chain length, and there are significant other minor constituents (see METHODS). To specifically address the role of saturated fats, we enriched



with stearate alone. Unfortunately, given weight loss despite the highest calorie intake, it seemed likely that the stearate diet was malabsorbed. We confirmed this by detecting a high fecal fat content in stearate-fed mice. Nevertheless, the weight loss, hypoinsulinemia, and high plasma corticosterone with stearate unexpectedly provided us with an insight into both the regulation of and an unexpected physiological role for elevated adipose 11 $\beta$ -HSD1 in fat mass regulation. Clearly, rather than promoting obesity and adipose insulin resistance (31) in this metabolic context, the strikingly elevated adipose 11 $\beta$ -HSD1 was associated with pronounced loss of fat mass. With fasting, glucocorticoids increase lipolysis by transcriptional upregulation of hormone-sensitive lipase (43) and the adipose tissue triglyceride lipase (desnutrin) in adipocytes (50) while suppressing lipoprotein lipase activity through induction of angiopoietin-like 4 (17). Our present work supports low insulin as permissive for the lipolytic effect of glucocorticoids (7) and further suggests that 11 $\beta$ -HSD1 may play a dominant role in this response locally in the adipose tissue. This concept is strongly supported by the finding that 11 $\beta$ -HSD1 $^{-/-}$  (28) and hexose-6-phosphate dehydrogenase H6PDH $^{-/-}$  (the enzyme that provides cofactor for 11 $\beta$ -HSD1-mediated glucocorticoid reactivation) mice (6) have lower fasting free fatty acid levels. The increased adipose 11 $\beta$ -HSD1 levels associated with weight loss in humans (46) are also consistent with a physiological role for elevated 11 $\beta$ -HSD1 in fat mobilization. Increased subcutaneous but not mesenteric adipose 11 $\beta$ -HSD1 precedes significant weight loss. Conversely, if this scenario is correct, high-fat-mediated downregulation of adipose 11 $\beta$ -HSD1 should attenuate glucocorticoid-mediated lipolysis and promote fat mass gain. It follows that downregulation of adipose 11 $\beta$ -HSD1 in response to high fat can only be considered beneficial if, by sequestering lipid away from muscle and liver, it prevents a state of more profound insulin resistance (4, 48).

High adipose 11 $\beta$ -HSD1, insulin, and free fatty acid levels coassociate in human and rodent (monogenic) obesity (31). In this case, increased adipose mass and, therefore, obesity may be maintained despite increased 11 $\beta$ -HSD1-dependent lipolytic activity because the adipocyte lipogenic pathway remains selectively sensitive to the high circulating insulin levels (4, 48). Furthermore, 11 $\beta$ -HSD1 (glucocorticoid)-dependent stimulation of de novo lipogenesis (6, 54) and lipid uptake from diet-derived chylomicrons through lipoprotein lipase (33, 35) will favor a net increase in fat mass. Selective hyperinsulinemia-driven lipogenesis (4, 48) may work in concert with the attenuated 11 $\beta$ -HSD1-mediated lipolysis to drive exaggerated adipose lipid accumulation.

Superficially, this analysis of the in vivo role of 11 $\beta$ -HSD1 in adipose tissue contradicts some of our previous in vitro findings. We showed that total 11 $\beta$ -HSD1 gene deficiency led to insulin sensitization of adipocytes (29), which indeed led us to believe that adipose 11 $\beta$ -HSD1 downregulation in DIO would be directly beneficial in this tissue (30). However, this was based on evidence of enhanced glucose uptake (29), consistent with the effect of glucocorticoids to impair glucose uptake in adipocytes (7, 40). Downregulation, deficiency (29), or pharmacological inhibition of adipose 11 $\beta$ -HSD1 clearly protects against this manifestation of insulin resistance and also appears to promote fat oxidation and suppress lipogenesis (3). However, the contribution of the lipolytic effects of glucocorticoids within the context of pathway-selective insulin resistance (4, 48) has not been elucidated, and the contribution of fat oxidation to overall fat mass is small (12). Our current data suggest that suppression of glucocorticoid (11 $\beta$ -HSD1)-mediated lipid mobilization could be a major physiological and depot-selective driver of increased fat mass. Consistent with this, 11 $\beta$ -HSD1 $^{-/-}$  mice accumulate fat preferentially in metabolically protective nonvisceral depots (29) where the enzyme is most highly expressed (30), reflecting the increased sensitivity of subcutaneous and visceral depots to insulin and glucocorticoids, respectively (1, 37, 38, 55).

The question of what mediates dietary fat regulation of adipose 11 $\beta$ -HSD1 is important. Likely candidates include catecholamines (stearate-induced weight loss), leptin, and the direct actions of the free fatty acids (or their metabolites). However, we have been unable to show any direct regulation of 11 $\beta$ -HSD1 in differentiated mouse adipocytes (3T3-L1) across a range of relevant concentrations of these factors (CL316242 1–100 nM, 1–100 nM leptin, 0.5–500  $\mu$ M stearate, palmitate, and oleate; unpublished observations). This leaves corticosterone and insulin, which show strong relationships with adipose 11 $\beta$ -HSD1 in the current study, as prime regulatory candidates.

Regulation of adipose 11 $\beta$ -HSD1 by insulin is a contentious issue and is influenced by dose, diet, and degree of adiposity (2, 32, 41, 51, 52). Although correlative, our results suggest that insulin acts as a negative regulator of adipose 11 $\beta$ -HSD1 under these in vivo dietary conditions. This fits with observations of reduced insulin and elevated adipose 11 $\beta$ -HSD1 in humans undergoing weight loss (46). Therefore, elevated adipose 11 $\beta$ -HSD1 in obesity may reflect resistance from a suppressive effect of insulin. Similarly, glucocorticoids can inhibit (2, 32) or increase 11 $\beta$ -HSD1 activity (6, 47) in vitro. In vivo, glucocorticoid administration increases adipose 11 $\beta$ -HSD1 (2, 25), whereas adrenalectomy reduces the elevated adipose 11 $\beta$ -HSD1 levels in obese Zucker rats (21). The positive correlation between corticosterone and adipose 11 $\beta$ -HSD1, accentuated by pair-feeding, suggests that it is a predominant (positive) regulator of adipose 11 $\beta$ -HSD1 levels when insulin action is low. Accordingly, elevated adipose 11 $\beta$ -HSD1 is found in genetically obese rodents with adipose insulin resistance (low insulin action) and high circulating corticosterone (21). Finally, association of pronounced visceral fat 11 $\beta$ -HSD1 activity in obesity (6, 9, 21, 26) may be due to this depot being intrinsically less sensitive to insulin (1, 55) and having higher levels of GR (24, 37, 38). In agreement with a depot-specific and insulin/glucocorticoid-sensitive role for 11 $\beta$ -HSD1, we found that shorter exposure to stearate and 45HF diets did not affect visceral mesenteric adipose 11 $\beta$ -HSD1 activity but did differentially affect activity in subcutaneous fat. The lack of an early effect in mesenteric fat suggests that a longer exposure with consequent effects on body weight, insulin, and corticosterone is required to change 11 $\beta$ -HSD1 activity in this depot. In contrast, in subcutaneous depots that are more responsive to insulin, changes in 11 $\beta$ -HSD1 activity are rapid and may immediately drive altered fat mass. Similarly, increases in hypothalamic 11 $\beta$ -HSD1 activity have been noted within 2 days of exposure (10), with possible implications in appetite regulation. It is notable that these early diet-induced changes in 11 $\beta$ -HSD1 in subcutaneous adipose tissue and hypothalamus precede any effects on body weight. Therefore, 11 $\beta$ -HSD1 may be key in driving body weight change at several levels.

Intriguingly, pronounced induction (stearate: high corticosterone, low insulin) or suppression (58HF: high insulin, low corticosterone) of 11 $\beta$ -HSD1 activity occurs despite more muted effects on 11 $\beta$ -HSD1 mRNA. Similar discrepancies between 11 $\beta$ -HSD1 activity and mRNA levels have been noted in intact visceral adipose preadipocytes (9) and in human visceral adipose tissue (13). Our current data suggest that a posttranscriptional mechanism is engaged at the extremes of physiological weight change that accentuates the effects on local glucocorticoid generation.

## Conclusions

Our dietary interventions strongly implicate adipose 11 $\beta$ -HSD1 in the regulation of fat mass, which is likely subject to the opposing influence of glucocorticoids and insulin (44). Tissue GR and plasma corticosterone levels are of lesser importance in this dynamic physiological regulatory mechanism. In contrast to its pathophysiological role in obesity (31), high adipose 11 $\beta$ -HSD1 likely facilitates mobilization of adipose triglycerides in the presence of low insulin/weight loss. Contrary to our previous view, the main effect of diet-induced,

adipose-specific 11 $\beta$ -HSD1 downregulation may be to promote fat accumulation (depot selectively), which, although initially protective of other metabolic tissues, ultimately leads to obesity and may worsen metabolic outcomes.

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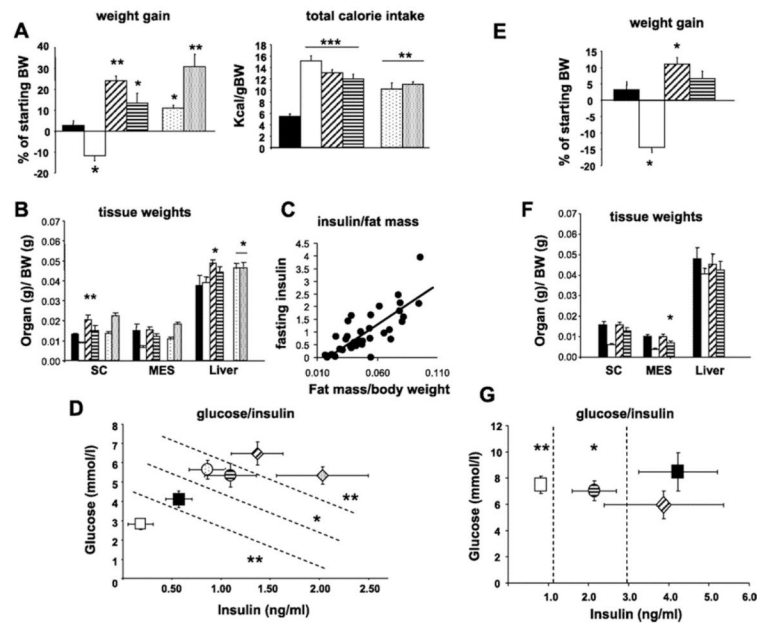
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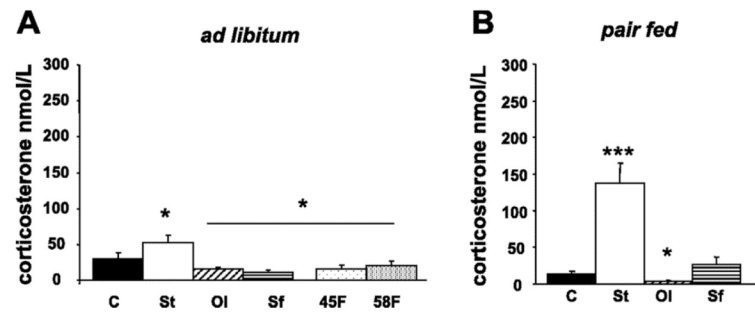


**Fig. 1.**

The effects of dietary fat enrichment on food intake, weight gain, and glucose homeostasis.

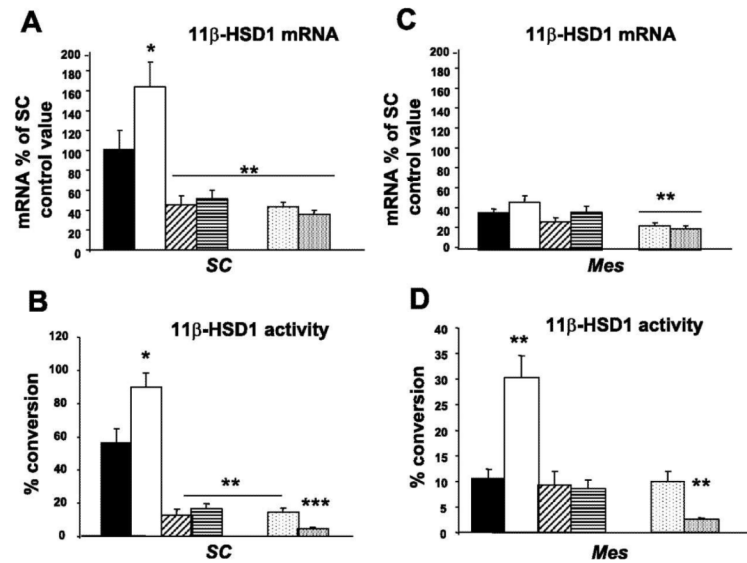
A: weight gain (left) and total calorie intake (right) after 4-wk ad libitum feeding with control (black bars), stearate (open bars), oleate (diagonal hatched bars), safflower oil (horizontal striped bars), 45HF (45% calories as fat; lightly stippled bars), and 58HF (58% calories as fat; heavily stippled bars) diets. B: changes in subcutaneous (sc) and mesenteric (MES) adipose and liver following dietary enrichment, as in A. C: linear regression of fasting insulin and combined fat mass [sc and MES, corrected for body weight (BW)] in all ad libitum-fed groups. D: fasting glucose/insulin ratio after ad libitum feeding of diets. Diagonal dashed lines represent significance boundaries relative by distance from the control diet mean values (■). Symbol infills indicate the same diets as the bar infills from the ad libitum-(A and B) and pair-fed (E and F) studies, respectively, and represent stearate (□), oleate (diagonal hatched diamond), safflower oil (horizontal striped circle), 45HF (lightly stippled circle), and 58HF (heavily stippled diamond).

E: weight gain after 4 wk of pair-feeding of the control and single fatty acid-enriched diets. F: changes in fat depot and liver weight after pair-feeding of diets as in C. G: morning glucose/insulin levels after pair-feeding of control and single fatty acid-enriched diets. Vertical dashed lines represent significance boundaries relative by distance from the control diet mean values (■). Symbols represent stearate (□), oleate (diagonal hatched diamond), and safflower (horizontal striped circle). Data are means  $\pm$  SE;  $n = 6/\text{group}$ . \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , significantly different from control diet. Note that the reduction in fat mass in the stearate groups is highly significant in all white fat depots, but annotation has been omitted from the graph for clarity.



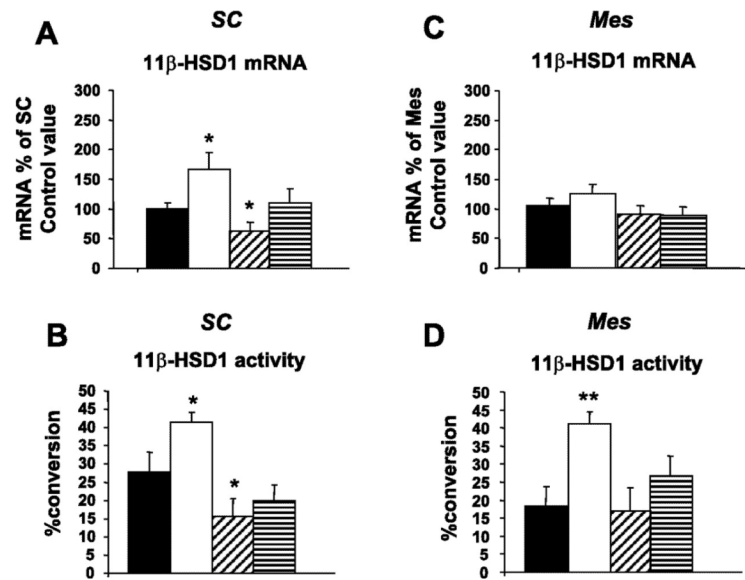
**Fig. 2.**

The effects of dietary fat enrichment on circulating corticosterone levels. A: corticosterone levels after 4-wk ad libitum feeding with control (black bars), stearate (open bars), oleate (hatched bars), safflower oil (horizontal striped bars), 45HF (lightly stippled bars), and 58HF (heavily stippled bars) diets. B: corticosterone after 4 wk of pair-feeding of the control and single fatty acid-enriched diets. \* $P < 0.05$ , \*\*\* $P < 0.001$ , significantly different from control diet.



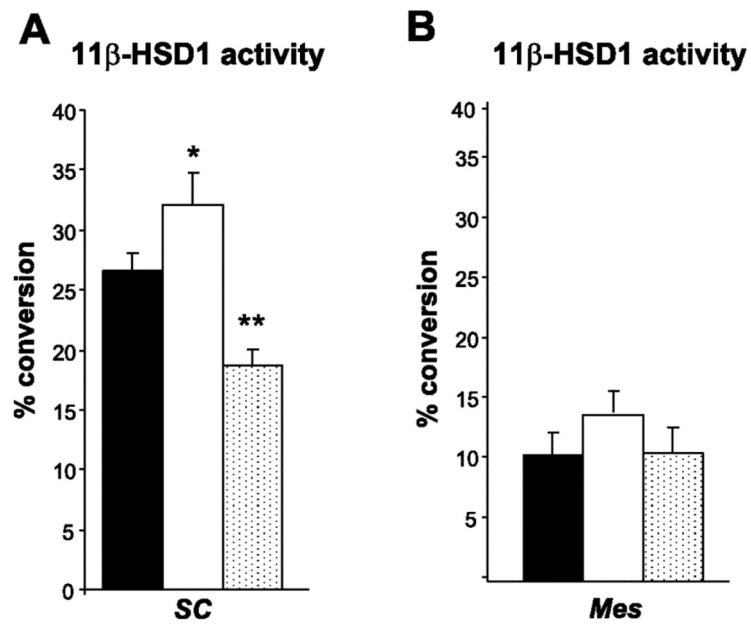
**Fig. 3.**

The effects of dietary fat enrichment on adipose 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) mRNA and activity levels. Adipose 11 $\beta$ -HSD1 mRNA (A and C) and activity levels (B and D) in sc (A and B) and MES (C and D) adipose tissues after 4-wk ad libitum feeding with control (black bars), stearate (open bars), oleate (hatched bars), safflower oil (horizontal striped bars), 45HF (lightly stippled bars), and 58HF (heavily stippled bars) diets. Data are means  $\pm$  SE; n = 6/group. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, significantly different from control diet.



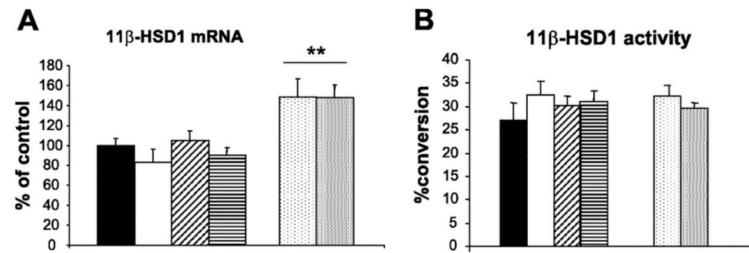
**Fig. 4.**

The effects of dietary fat enrichment with pair-feeding on adipose 11β-HSD1 mRNA and activity levels. Adipose 11β-HSD1 mRNA (A and C) and activity levels (B and D) in sc (A and B) and MES (C and D) adipose tissues after 4 wk of pair-feeding control (black bars), stearate (white bars), oleate (hatched bars), and safflower oil (horizontal striped bars) diets. Data are means  $\pm$  SE; n = 6/group. \*P < 0.05, \*\*P < 0.01, significantly different from control diet.



**Fig. 5.**

The effects of short-term (1 wk) stearate-enriched or 45HF diet on adipose 11β-HSD1 activity levels. A: sc adipose tissue 11β-HSD1 activity after 1 wk of control (black bars), stearate (open bars), or 45HF (lightly stippled bars). B: MES adipose tissue 11β-HSD1 activity after 1 wk of control (black bars), stearate (open bars), or 45HF (lightly stippled bars). Data are means  $\pm$  SE;  $n = 6/\text{group}$ . \* $P < 0.05$ , \*\* $P < 0.01$ , significantly different from control diet.



**Fig. 6.**

The effects of dietary fat enrichment on hepatic 11β-HSD1 mRNA and activity levels. A: liver 11β-HSD1 mRNA. B: activity levels after 4-wk ad libitum feeding with control (black bars), stearate (open bars), oleate (hatched bars), safflower oil (horizontal striped bars), 45HF (lightly stippled bars), and 58HF (heavily stippled bars) diets. Data are means  $\pm$  SE; n = 6/group. \*\*P < 0.01, significantly different from control diet.